Influence of *Hydractinia echinata* on the shell-choice of the hermit crab *Pagurus bernhardus*

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Abstract
Pagurus bernhardus is a hermit crab, which is prone to predators and injuries, due to its scarcely sclerotized abdomen. Its survival depends on the availability of the shells of gastropods, e.g. the common periwinkle *Littorina littorea*. Hermit crabs do not enter shells at random. They choose the new shell according to criteria such as width, height, weight and centre of gravity. Hermit crabs are often found associated with the epibiotic polyp *Hydractinia echinata*. The present study aims to answer the question, whether *P. bernhardus* shows a preference for shells that are encrusted with the polyps or if they rather pay attention to other criteria. To answer this question 15 hermit crabs were experimentally isolated from their shells and tested in a two alternative forced choice paradigm (2AFC). They were offered one shell without *H.echinata* and one encrusted with the polyps and their choice behaviour was observed for 90 min. The results present evidence that *P. bernhardus* does not select shells according to the encrustation with *H. echinata*, suggesting that the animals rather pay attention to other criteria. It is yet to be determined, which parameters are the most important ones, when making a shell choice.

Introduction

Pagurus bernhardus L. is the only hermit crab species in the Wadden Sea, where its primary habitats are the subtidal channels in which shell gravel and mussel beds occur (Reise, 2004)]. Hermit crabs are decapod crustaceans of the family Paguridae within the superfamily Paguroidea (Hazlett, 1981). During spring and autumn the juveniles and young adults migrate to the lower tidal zone and retreat or hide in sheltered places with each falling tide. During winter they migrate to greater depths, whereas large individuals remain subtidally the whole year through (Reise, 2012)

Just like most other hermit crab species, *P. bernhardus* has a long spirally curved abdomen, which is very sparsely sclerotized (Gravel et al., 2004). Therefore they are prone to injuries and capture by predators unlike other related crustaceans. That is the reason why survival is heavily dependent on the availability of suitable shells from e.g. sea snails as a main defence mechanism against predators and the physical hardships a life in the intertidal zone entails.

Due to their growth or damage of their own shells, hermit crabs are in constant need of new or larger shells. They do not enter shells at random, but select them according to criteria such as e.g. height, shape, opening width, weight (Davis & Smith, 2007) and centre of gravity (Reese, 1962). To estimate the quality of a shell they use a variety of olfactory, sensory and visual information, e.g. drumming against the shells side with their claws to gain knowledge about the condition, shape and size (Reese, 1962).

In the Wadden Sea *P. bernhardus* often resides in empty shells of the common periwinkle (*Littorina littorea*), which is often associated with the marine colonial hydroid *Hydractinia echinata*, also known as snail fur. It is speculated that the hydrozoan colony continuously enlarges the hermit‘s shell by building on the lip of the shell and guarding the fringe of the shell with spiralzoids, thus protecting the hermit crab from predation (Davis, M. & Smith, 2007). Further advantages might be a strengthened shell (Statchowitsch, 1980), increased crypsis (Ross, 1971) and tolerance to desiccation. The increased external diameter might be advantageous in matters of shell-crushing predators. On the other hand the internal volume-weight ratio decreases with increased thickness of the shell, thus making it more difficult to carry (Briffa & Elwood 2005). This is likely to increase energy costs. Much of the available evidence suggests that the association with *P. bernhardus* is more advantageous for *H. echinata* than it is for the hermit crabs (Williams & McDermott, 2004). The polyps profit from the transport into nutrient rich waters by the hermit crab.

We conducted experiments to address the question, whether P. bernhardus has preferences for un-encrusted shells from L. littorea or commensurate shells that were covered with H. echinata.

Material and Methods

Sampling

Shells of *L. littorea* with *P. bernhardus* and encrusted with *H. echinata* and empty un-encrusted shells were collected in the North-Eastern Wadden Sea on the left and right beachsectors of the harbour of Sylt, List (Germany) at low tide between the 25th and 27th September. Furthermore clean empty shells of different size of *L. littorea* and 7 living snails of bigger sizes were collected. The trials were conducted at the AWI – Alfred Wegener Institut, Helmholtz-Zentrum für Polar- und Meeresforschung, located in List.

In total 26 hermit crabs, living in shells covered with H. echinata, were collected, 16 of them were used in the trials. They were held in open 30x 40x 4 cm plastic-tanks with constant seawater flow, sand and one opened common blue mussel *Mytilus edulis*. 53 empty shells of *L. littorea* and living common periwinkles were collected and measured with a calliper (accuracy +/- 0,01) in height, width and opening width. The shells of the hermit crabs were evaluated in the same matter.

To estimate whether the shell height is a suitable measure for the size of the shell, we tested if the width and the height of all measured shells were correlated.

Statistics

The ratio of height to opening height of colonized and clean shells were compared using a paired t-test with unequal variances. A p-value $\leq 0,05$ was accepted as significant and is depicted as an asterisk (*). The linear regression (Fig. 2) was done with Excel 2010. The height and the width of the shells were correlated and the slope and the R^2 were calculated (Fig. 2).

Experimental Design

Shell-pairs (one shell with and one without the polyps) with commensurate shell-sizes, similar to the current shell, were generated for each crab. Only intact, undamaged shells were used for the experiments.

Because there were not enough empty shells of the required size found, 7 snails had to be cooked in boiling water for 2 min. and pulled out of their shells. Empty shells were assigned to each hermit crab, depending on the height of the current shell (with a variance of ±0.2 cm). The occupied shells colonized by the polyp were allocated to commensurate hermit crabs, depending on the height of the current shell.

The hermit crabs were forced out of their shells with approximately 35 °C 2:3 fresh water / seawater. Preliminary tests were conducted with 3 hermit crabs occupying netted dog-whelks (Hinia reticulata) to determine if the hermit crabs and polyps can cope with the treatment. By breaking the shells with a bench vice (Briffa & Elwood, 2005) the stress level would have been much lower but the encrusted shells could not have been used in the trial. Since no empty shells encrusted with *H. echinata* were found, the hot

Fig.1. Scheme of the experimental design (right) and photograph of the actual trial setup (left).

fresh water treatment was the best option.

During the actual trial the ratio of fresh water to seawater was adapted to 1:3 seawater to fresh water, since the crabs would not come out by itself fast enough and hermit crabs did not cope well with longer fresh water treatment. After pulling them out of their shells the hermit crabs were acclimatized in salt water for approximately 1 hour.
Experiments were aborted, when they did not come out of their shell fast enough and the crab was excluded from the trial.
The trials were conducted in white polyethylene arena with a diameter of 15.6 cm and 4 cm height. For better hold the arenas bottoms were covered with sediment and filled with 3 cm seawater. All trials were conducted simultaneously to control for factors like day time, light and temperature. After 90 min we defined the shell-choice of the hermit crab as final. Any other choices that might have occurred beyond that timeframe were disregarded. The first choice the hermit crabs made was noted. We started noting the choice in ten minute interval 30 min into the trial. The animals were confronted with the pair of shells in a two alternative forced choice paradigm (2AFC). The shells were presented in 2 cm distance from the hermit crab in juxtaposition with each other (Fig. 1). Directly after the experiments all animals that recovered sufficiently were brought back to the sea.

Results

Ratio of Height to Opening Height

The method for removing the crabs from the shell seemed to be very stressful since 7 crabs did not survive the treatment. Six of them could not be pulled out of the shell at all. It was not clear, if they could move out on their own. It seemed that the openings of the encrusted shells were much smaller than the ones of un-encrusted shells. This observation was confirmed by a paired t-test of the opening heights with unequal variances, depicted in figure 3. The height to opening height ratio was compared between encrusted and un-encrusted shells. For un-encrusted shells we observed an average ratio of 1, 51 cm (s.e.m. 0, 06 cm). In case of the encrusted shells the ratio was significantly larger (2, 01 cm ± 0, 07 cm) as can be seen in figure 2.

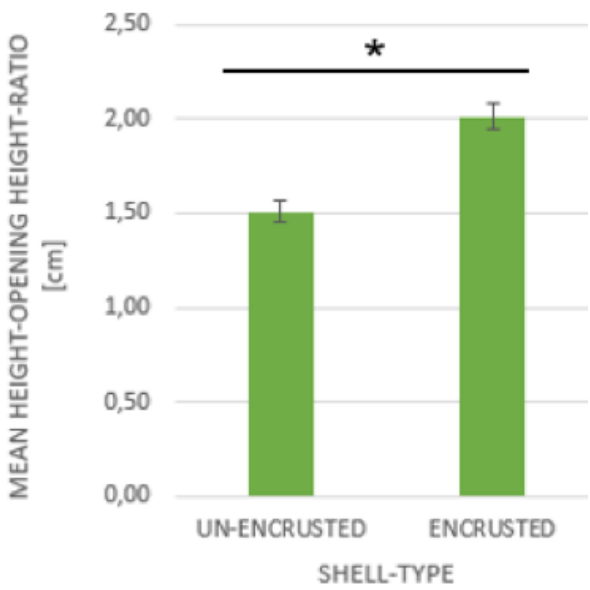

Fig. 2. Mean Height-opening height ratio in cm as a function of un-encrusted or encrusted shells. Error bars are depicted as s.e.m. A paired t-test with unequal variances was conducted to compare both ratios. (p-value < 0,05*).

Correlation between Shell-Height and –Width

To estimate whether the shell height is a suitable measure for the size of the shell, it was tested if the width and the height of all measured shells were correlated. The results presented in figure 3 show a linear correlation that can be estimated as $y = 0{,}5579x + 0{,}3736$ with $R^2 = 0{,}6219$.

Shell-choice of *Pagurus bernhardus*

The shell-choice of *P. bernhardus* was observed in the arena-experiments described in Material & Methods. The results are presented in figure 4. Out of 15 hermit crabs that were analysed in these experiments 8 chose the clear shell, whereas 7 crabs were located in encrusted shells at the end of the experiments.. Five of the 15 crabs changed shells during the trial and 3 out of 15 changed continuously and did not seem to be able to make a decision. They examined the empty shells extensively before moving in. Interestingly, six crabs did

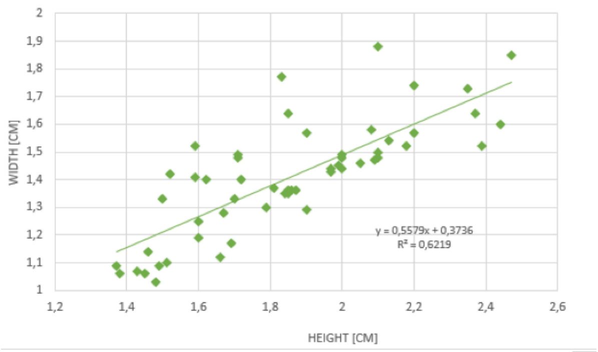

Fig. 3. Correlation between shell width and height in cm. Linear regression was done in Excel 2010.

not enter any shell for 30 min., two of them did not even enter any of the shells until 60 min after the beginning of the trial.

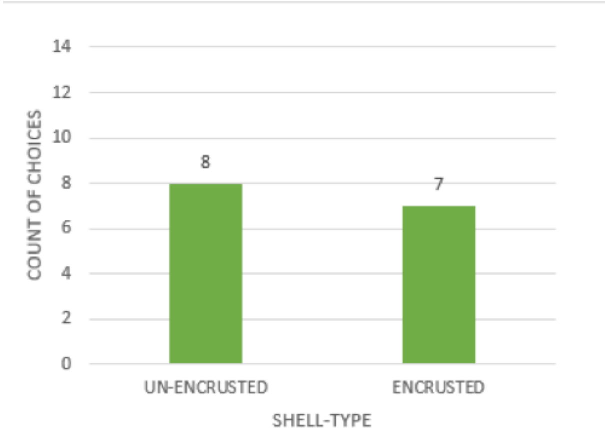

Fig. 4. Number of choices observed for un-encrusted und encrusted shells.

Discussion

The shell-choice behaviour observed in the hermit crabs experimentally isolated from their shells *P. bernhardus* shows no apparent preference for *L. littorea* shells encrusted with the epibiotic polyps *H. echinata*. It is not clear, if they were in a poor condition after the treatment with fresh water or if there were other reasons for that.

For immediate protection it would be better for the hermit crab to choose shells with a lower contrast compared to the substrate, since these shells are harder to detect for predators, e.g. birds. But shells with a higher contrast might also be better detectable for the crabs. *H. echinata* forms light, slightly pink patches on the gastropod shells, hence enhancing the crypsis by making them appear paler in contrast to the substrate than shells without the polyps. Briffa & Elwood (2005) found that epizoic fauna might increase the protection from predators but might also decrease the shells quality by increasing the weight the hermit crabs have to carry. Since we used sand to cover the bottom of the arena, the contrast between substrate, new, and current shell should not differ greatly from their natural environment. To rule out preferences linked with the contrast, the same colour could have been used for all shells in the experiments (Briffa et al., 2008).

It is widely known that hermit crabs like *Dardanus* spp. live in close relationship with their symbionts and sometimes even actively transfer their epizoic fauna (Caruso et al., 2005). But it is also known that *P. bernhardus* does not show any behavioural patterns concerning their epibionts (Ross, 1971). It is not clear, whether P. bernhardus pays attention to epibionts at all. Calcareous epibionts like barnacles or polyps that cling to the shell, cannot be moved (Ross, 1971) and it was not observed in this study. It seems more likely that experimentally isolated hermit crabs rather pay attention to the first shell that offers protection. To rule out any interaction with other epibionts the shells could have been cleaned from any other epibionts such as barnacles and algae.

Briffa & Dalaway (2007) showed that males and females display different fighting behaviour over shells and it might be possible that they also make a different shell choice due to different criteria.

Gravel et al. (2004) proposed experiments to keep the variable choice dimensions from differing between two shell-choice options. They designed identical, artificial shells that rule out any form of natural variability and tested for the animals preferences. They focused on height width and other parameters but did not investigate the role of *H.echinata*. The hermit crabs and snail shells used in the present study were collected in the field, and only a limited timeframe was available for the execution of the experiments, hence it was not possible to eliminate all other parameters that influence the hermit crabs choice behaviour. The offered shells were about the same size and quality but still differed slightly in one or more dimensions (e.g. shell thickness, weight, colour and other epibionts).

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